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Differential Responses of Soybean Foliage to Simulated Acid Rain[1]

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Abstract : The primary objectives were to determine the acidic rain stress cause alternation in the soybean foliar injury responses. potted soybean (*Glycine max*.L. cv. "Tainung 15" and "Kaohsiung select 10") were exposed to two 1 mm simulated rain episodes per week at specific acidities from pH 2.0, 3.0, 4.0 and 5.6 treatments. Each treatment was exposed to 0.5hr spraying in the morning between 8 : 00 and 9 : 00 on consecutive days for 5 weeks after anthesis (R2) stage. Acid rain treatment at pH 2.0 induced the formation of pale brown necrotic spots on the leaves within 2 weeks after exposure. The electrolyte leakage of the first trifoliate leaf to acid rain of pH 2.0 for 1 week was significantly greater than that from the other treatments. cuticular stomata exposed to the acid rain of pH 2.0 showed a smaller aperature and did not follow diurnal changes. However, acidity of rain had no effect on stomatal conductance of the soybean. After 2 weeks of exposure, in pH 2.0 and pH 3.0 treatments, soluble protein and chlorophyll content of "Tainung 15" showed a significant decrease. However, "Kaohsiung selection 10" was not significantly affected by pH 3.0 episode.

Key words : Acidic rain, Soybean, *Glycine max* L., Electrolyte leakage, Cuticular conductance.

INTRODUCTION

Acid precipitation has often been defined as precipitation with pH values less than 5.6 [2,12]. A pH of approximately 5.6 is the equilibrium pH for the dissolution of ambient atmospheric level of carbon dioxide (approximately $320\mu LL^{-1}$) in water, a reaction that produces carbonic acid. Measured pH values of precipitation in Taiwan were often lower than 5.6, because of the large amount of sulfur and nitrogen oxides in the atmosphere.

There have been many reports on the occurrence of visible injury induced by acid rain treatment. Since Shriner et al. (1974) first reported the findings of their acid rain study. Jacobson (1980) reported that the threshold for foliar injury development was among pH3.0 and pH 4.0. Hosono and Nouchi (1994) found that simulated acid rain at pH 3.0 or below produced visible injury on leaves of six species. Bean leaves exposed to acid rain from

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sulfuric acid at pH 3.0 and below showed necrotic spots[9]. Kohno and Kobayashi (1989) showed that simulated acid rain treatment at pH 3.0 or higher did not induce significant adverse effects on the growth of soybean seedlings, but acidic rain treatment at pH 2.0 significantly reduced leaf area, dry matter accumulation and root nodule prodution.

Acid rain experiments on agricultural crop species in Taiwan have not been investigated to any extent. The aim of this study is to measure the physiological response and leaf injury of *Glycine max* after exposure to simulated acid rain solutions under greenhouse conditions.

MATERIALS AND METHODS

Plants material and growing conditions

Soybean seeds ["Tainung 15" (TN15) and Kaohsiung select 10:(SSC10)] were sown in plastic pots (3 seeds to a pot, pot size: 18 cm in diameter x 25 cm in depth) filled with 2L of loamy soil. Seedlings were thinned to one plant per pot 7 days after seedling. Fertilizer containing N, P and K was applied to the soil at a rate of 80:100:100 kg ha^{-1} as $N:P_2O_5:K_2O$ prior to seeding. The experimental plants were grown located under greenhouse that served as ambient rain exclusion shelters for the delivery of simulated acid rain.

Aced treatments

The pH of the rain solution was adjusted to 5.6, 4.5, 3.0 and 2.0 with a mixture of H_2SO_4 and HNO_3(2.5:1). Solution in a PVC supply canister was pressurized with hand and forced through a small orifice of a commercial stainless steel rain nozzle. Where it impinged on the tip of a J-shaped pin, and was broken into particles with a mean stain diameter of 1.0mm (Fig. 1). Spraying was done carefully to wet the leaf surface evenly and completely, so that

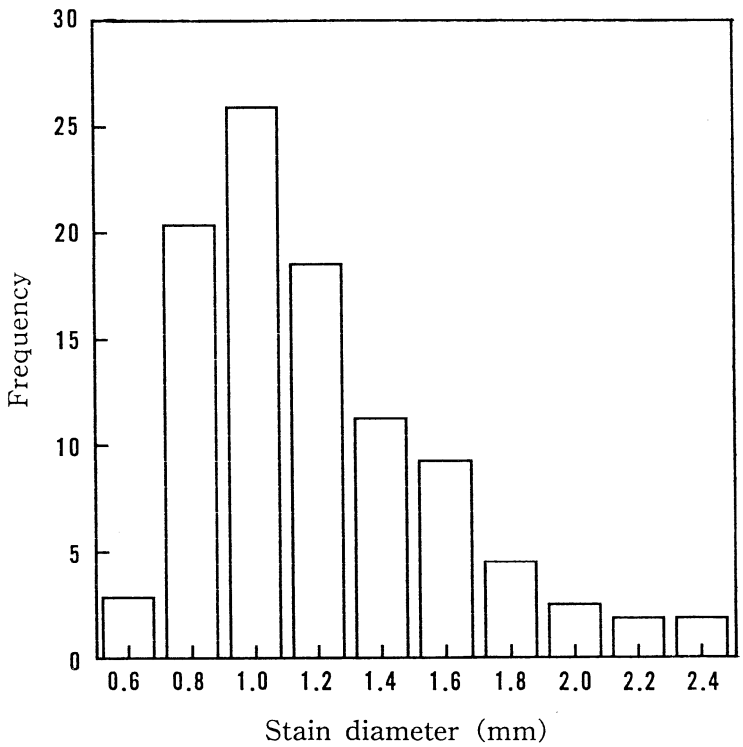

Fig. 1. Drop stain distribution for simulated acid rain exposure system.

no spray reached stems, petioles, soil surface, opposite leaves, or other parts of the plant. Each treatment was exposed to 0.5hr spraying in the morning between 8:00 and 9:00 twice a week on consecutive days for 5 weeks after anthesis (R2) stage (approximately R3-R6 stage, seed filling period).

Analysis

Chlorophyll and soluble protein: Measured on subsamples of fully expanded leaves from the upper half of four plants per treatment, six discs of 1.0 cm in diameter were taken from interveinal region of leaves. The measurement followed the method of Arnon (1949) and Bradford (1976).

Wax content : Twenty discs of 1.0cm in diameter were taken from interveinal region of leaves, epicuicular waxes were removed by stirring the leaves in 30ml chloroform for 15 seconds[13].

Electrolyte leakage : The leaves were placed in 50ml plastic tube and incubated at ambient temperature, conductivity of the suspending solutions was measured after 24 hrs and again after autoclaving at 121°C for 10 min (taken as 100 % electrolyte leakage).

Stomatal and cuticular conductance : Measured on the terminal leaflet of the fully expanded leaf with a steady-state porometer (Model LI-1600, Licor Crop., Lincoln, NB).

Relative water content (RWC): The fresh weight (Wf) was measured immediately after the leaf tissue was collected and the fully turgid fresh weight (Wt) was measured after the leaf tissue had been brought to fully turgor by floating the cut leaves in water for 2h. The dry weight (Wd) was determined by heating the leaf samples at 100°C until the weight no longer decreased. RWC is expressed as $RWC(\%)=100\times(Wf-Wd)/(Wt-Wd)$.

Ultrastructure of leaf surface : In R5 growth period, samples of exposed leaves were taken for investigation of the epicuticular wax layer by scanning electron microscopy.

Statistics

Analysis of variance were carried out on all data. Probabilities were determined using the standard Student's t-test. The data shown are means of 12-15 independent replicates ± SEM.

RESULTS

Acid rain treatment at pH 2.0 induced the formation of pale brown necrotic spots on the leaves within 2 weeks after exposure. Necrotic symptoms first apperared along the vein and spread gradually into the interveinal areas. When young leaves were exposed to acid rain at pH 3.0, only slight injury was observed in the form of necrotic spots, mature leaves showed no visible symptoms of acid rain injury at pH 3.0 and greater. However, necrosis was severe enough to result in abscission at pH 2.0. Abscission occurred after the initial exposure to pH 2.0, but plants initiated new laterals to continue vegetative growth. Lateral regrowth appeared to be less sensitive to injury than primary or secondary leaf tissue.

The electrolyte leakage of the first trifoliate leaf from soybean plant exposed to acid rain of pH 2.0 for 1 week was significantly greater than the from the other treatments (Fig. 2.). Compared to Tainung 15 exposed to acidic rain, Kaohsiung select 10 treated with pH 3.0 rain exhibited significantly higher electrolyte leakage levels. But, no difference was found in electrolyte leakage between pH4.0 and 5.6 even after 5 weeks exposure.

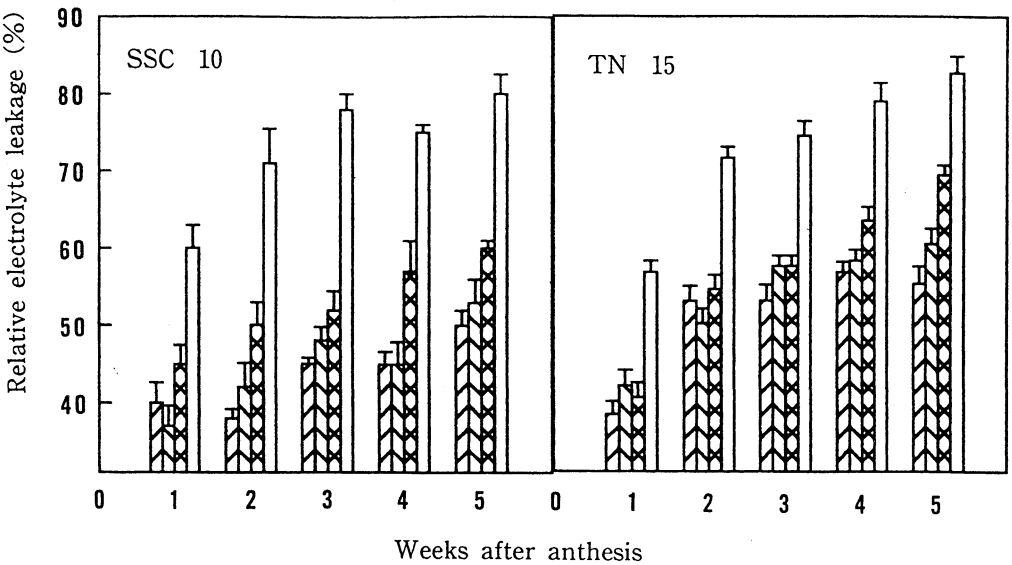

Fig. 2. Changes of relative electrolyte leakage of soybean leaf in simulated acid rain during seed filling period.
SSC 10 : c.v Kaohsiung select 10 TN 15 : c.v Tainung 15
Bars indicat standard error.

After 2 weeks of acid rain treatment, the cuticular conductance of leaf at pH2.0 was significantly less than that at pH 5.6, otherwise there were no significant difference among any of the three acid rain treatments. After 5 weeks of exposure, the cuticular conductance was not significantly different among four pH treatments (Fig. 3.). On September 17, daily time courese of cuticular conductance of leaves were measured. Cuticalar stomata exposed to the acid rain of pH 2.0 showed a smaller aperture and did not following diurnal changes (Fig. 4.). However, acidity of rain had no effect on stomatal conductance of the soybean (data not shown). At the end of the experimental period the quantity of leaf relative water content are shown in table 1. Relative water content was not significantly affected by acid rain treatments at pH 3.0 or higher; that at pH 2.0 was significantly less than that of the control at pH 5.6.

Fig. 5. shows the change of soluble protein of leaf exposed to different pH episodes after anthesis stage. After 2 weeks of exposure, in pH 2.0 and pH 3.0 treatments, leaf soluble protein of Tainung 15 showed a significant decrease. However, Kaohsiung select 10 was not significantly affected by pH 3.0 episode. The chlorophyll content of soybean leaves showed similar results with the change of soluble protein (Fig. 6).

Acid rain of pH 2.0 had a detrimental influence on the epicuticular wax layer which showed numerous lesions and melted appearance (Fig. 7.). In contrast, the epicuticular wax layer of the pH 5.6 is characterized by a dense stand of small threads. The quantity of cuticular wax was shown in Table 1. Damage symptoms of epicuticular stomata were observed in the leaf after application of acidic rain (Fig. 7.).

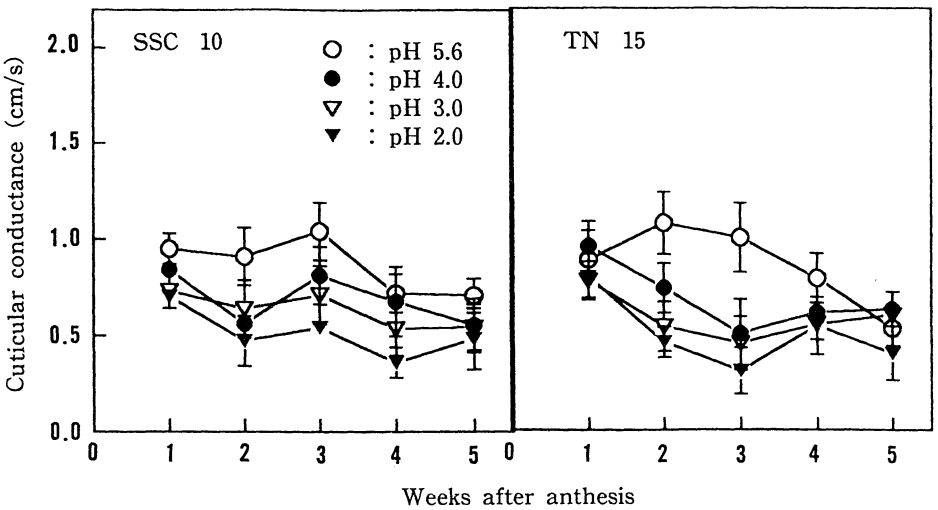

Fig. 3. Changes of cuticular conductance in the soybean leaf in simulated acid rain during seed filling period.
Bars indicat standard error.

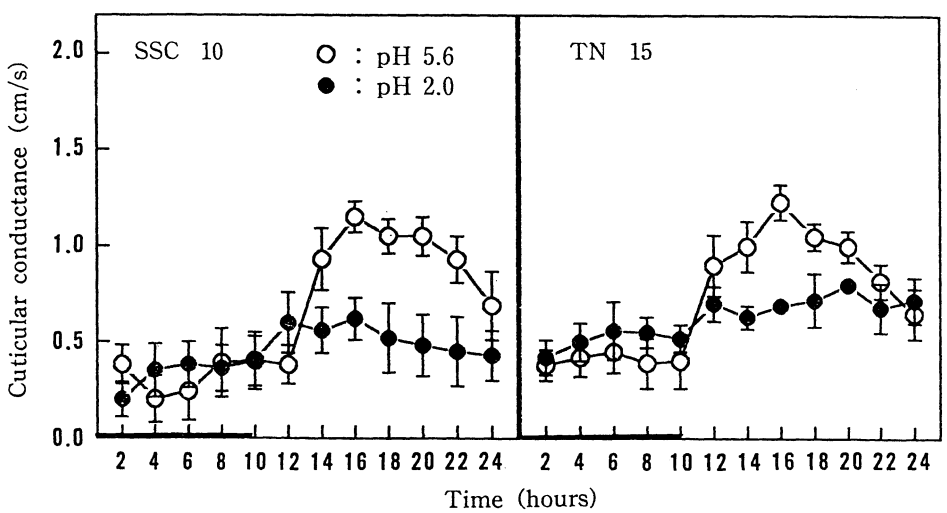

Fig. 4. Time-course-changes of cuticular conductance in the soybean leaf in simulated acid rain during seed filling period.
(september 17. mean temp. 25.6°C , relative humidity 78%) .
Bars indicat standard error.

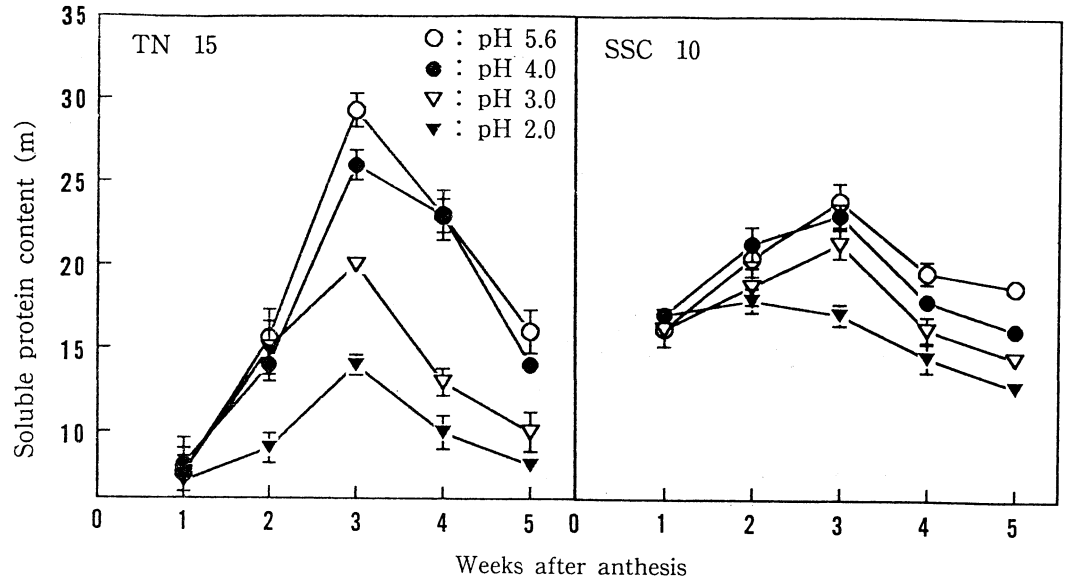

Fig. 5. Changes of soluble protein content of soybean leaf in simulated acid rain during seed filling period.
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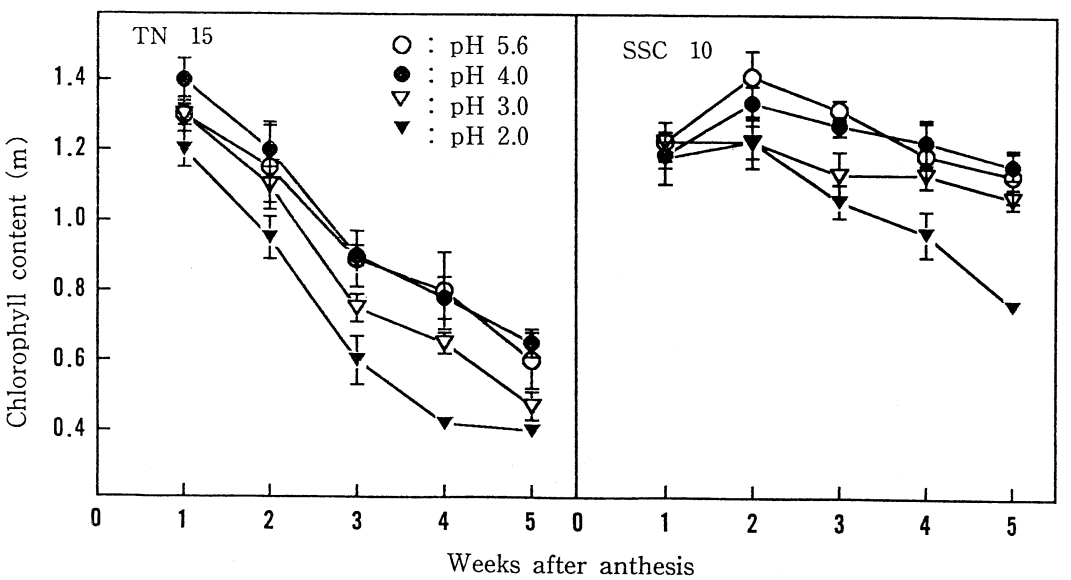
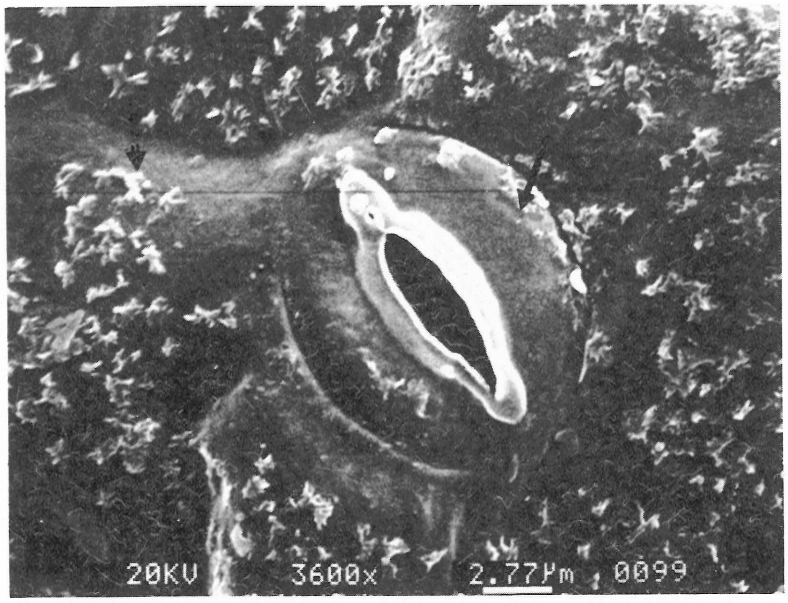


Fig. 6. Changes of chlorophyll content of soybean leaf in simulated acid rain during seed filling period.
Bars indicat standard error.

A



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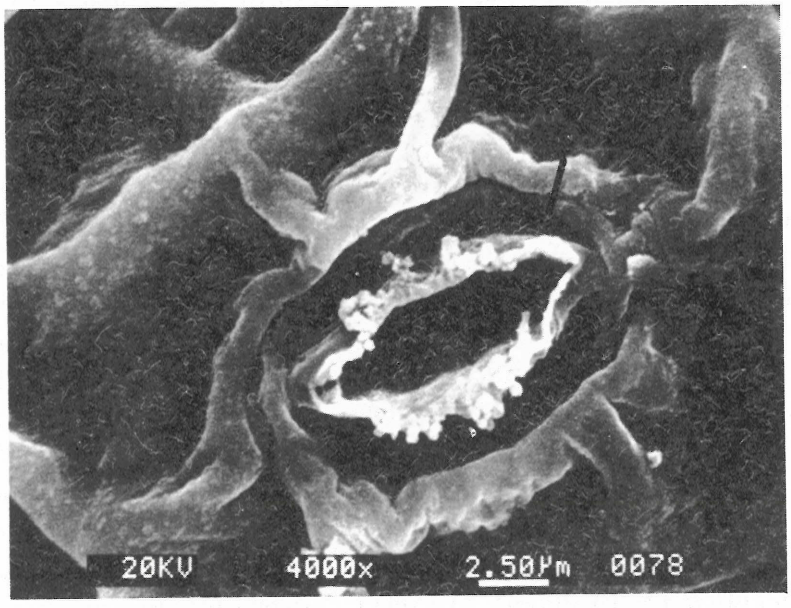

Fig. 7. Scanning electron micrographs of the stomata (solid arrows) and cuticular wax (dash arrow) structure in soybean leaf during two weeks exposure. A : After application of control rain (pH5.6) .B : After application of acidic rain (pH2.0)

DISCUSSION

The phytotoxicity of acidic rain is mainly due to the direct deposition of acidic compounds on plant surface, which can ultimately result in structural injury[3,14,20]. The effect of acidic rain on injury growth and yield has been determined for a wide variety of crops[17]. In soybean, plants are adversely affected only by pH levels of 3.0 or lower[22]. Injury (necrotic lesions) began to appear on leaves, stems and fruit at pH 2.8 or lower. More acidic rain of pH 2.0 or lower are required for growth or yield decreases[11]. According to Kobayashi et al. (1991), the exposure of simulated acid rain at pH 2.0 caused the reduction of dry weight growth and seed yield of soybean plant due to acute damage of leaf area growth. In our study, leaves of two cultivars were damaged by acidic condition at pH 3.0 or lower. The acid treatments of pH 2.0 seriously affect the leaves, causing bleached and necrotic lesions. Some of the photosynthesizing area of the leaves was damaged and chlorophyll was reduced or absent from these lesions. Tainung 15 exposed to pH 3.0 rain exhibited reduced chlorophyll content (compared to responses in plants exposed to pH 5.6 rain), but Kaohsiung select 10 was not significantly altered, With respect to other physiological changes, acidic rain effects on soluble protein responses were of similar magnitude to alternations in chlorophyll in the present study (Fig. 6.).

According to Tukey (1970), leaves continually excrete small amounts electrolyte molecules. Beginning at the lst week of rain application in soybean, leaching rates of the control plants were much lower than those of the plants exposed to pH 2.0 rain. The higher leaching rates in the acidic rain treatment may be explained by the detrimental effect of acidic rain on the cuticle. However, it is also possible that the protons of the acidic rain may have exchanged Ca^{2+} from the surface of the plasmamembrane. Mengel et al. (1990) reported that protons may exchange metallic cations from *Picea abies* leaves, in which, ions, particularly Ca^{2+}, were exchanged at the beginning of the exposure to acidic fog. As can be seen from Fig. 2., leaching rates increased with time. This increase was significant and indicated that the permeability of the membrane of the cuticle or both became higher.

The disintegration of the epicuticular wax layer brought about by acidic rain (Fig. 7, Table 1) has also been previously reported. In addition, our investigation provided evidence that the epistomatal wax layer was heavily damaged by acidic rain. This observation is in good agreement with the results of Mengel et al. (1989), who reported that acidic fog brings about a cracking of the epistomatal wax props.

According to Jeffree et al. (1971), the epicuticular waxes on the stomatal cavity of spruce provide excellent protection against water loss. Consequently damage or even loss of the epistomatal waxes will lead to a disturbance in the water status of plants. Trumble and Walker (1991) reported that stomatal resistance increased significantly in *Phaseolus lunatus* following exposure to pH 2.5 and pH 3.0 acidic fog. Damaged cuticle and guard cell should have and impact on the cuticular stomatal fluctuation of soybean, as were shown in our study. but Knittel and Pell (1991) reported that acidity of rain had no significant effects on the cuticular or stomatal conductance of the corn cultivar at pH 3.0 treatment. There are

several possible explanations for the apparent lack of response of cuticular conductance to acidic rain. Other cuticular stresses could mask effects of acidic rain. Wind, relative humidity, temperature, light intensity, and insects or leaf microflora could all affect cuticular conductance. in addition, This differential results were dependent upon plant species, leaf surface, characterisitics and amount of acid input.

A damaged cuticule should have and impact on the water holding capacity of plants, as was shown in this paper. Soybean with a degraded epicuticular wax layer are susceptible to water stress and their protection mechanism against water loss is affected since even with stomatal closure water may be lose by cuticular transpiration. It can there be assumed that the decline of spruce trees widely occuring in central Europe could to a large extent be caused by cuticle damage that leads to water stress, particularly in dry years[18,19].

Moskowitz et al. (1985) estimated that if acid deposition in the United States were reduced by 10% in 1982, the yield of field grown soybean cultivars would have been greatly increased. However, they suggested that the effects of acid precipitaiton might be less important than those of dry deposition such as O_3 and other environmental stress such as water stress. Thus, further research is needed to assess long term interactive effects of acidic precipitation and other environmental factors on soybean and othere agricultural crops.

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人工模擬酸雨對大豆葉片之不同反應[1]

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摘要：本試驗目的主要是探討酸雨對大豆葉片傷害。塑膠溫室內以盆缽方式種植大豆，於開花期後（R3）開始噴施不同酸度（pH2.0，3.0，4.0和5.6）的雨水，每週噴施1公厘雨量2次，連續5週。試驗結果顯示，pH2.0酸雨噴施葉片2週後，出現白色及褐色斑點，pH3.0有少量斑點出現，至於pH4.0及5.6則不受影響。葉片電解質滲漏量於pH2.0噴施1週後迅速增高，顯示葉片細胞受到傷害。強酸使表層導度下降且無日變化，氣孔導度則各處理間並無差異。「臺農15號」葉片之可溶性蛋白質和葉綠素於 pH2.0及3.0處理2週後開始下降，「高雄選10」號則祇有 pH2.0處理有下降趨勢。

關鍵詞：酸雨、大豆、電解質滲漏量、表層導度。

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